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RESEARCH ARTICLE

DETERMINATION OF ORGANOCHLORINE PESTICIDE RESIDUES IN THE WATER OF PADDY FIELDS OF PRAKASAM DISTRICT, ANDHRA PRADESH, INDIA

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ABSTRACT

Organochlorine (OC) pesticide residues in pooled water of paddy field samples of Prakasam District have been investigated to find out the extent of pesticide contamination and accumulation. Collected Water samples were processed using a liquid-liquid extraction followed by Gas-Chromatographic technique for pesticide residue quantification with mass selective detector (GC-MS). The conditions were followed for GC, Column: HP-5MS, 30m x 0.25mm x 0.25 µm 50% Phenyl Methyl Siloxane, Injection Temp: 260 ^{0}C, Injection Vol. 1µl, Column flow: Helium 1mL/min, Oven hold up time 2.0 min. For MS, Mass spectrometer with single Quadrupole, Operation in SIM Mode, MS Source:230 ^{0}C, MS Quad: 150^{0}C Solvent Delay: 3.00 minutes and GC-MSD detecter were maintained. Eight OC pesticides namely α-HCH, β-HCH, δ-HCH, γ-HCH, Dieldrin, β-Endosulfan, p,p-DDD and o,p-DDT were traced. In the pesticide residues, highest β-Endosulfan (0.000082 mg/L) followed by γ- HCH (0.000418 mg/L), o,p-DDT (0.000228 mg/L), α-HCH (0.000082 mg/L), δ-HCH (0.000067 mg/L), p,p-DDD (0.000062 mg/L), β-HCH (0.000040 mg/L) and Dieldrin (0.000025mg/L) were traced in analyzed water samples. The LOD and LOQ were calculated as 0.000006 mg/L and 0.00002 mg/L.

INTRODUCTION

To meet the demands of the growing population it is necessary to increase the food production. The introduction of high yielding varieties has necessitated the farmers to use fertilizers and pesticides for the control of insect's pesticides. However, improper use of pesticides has resulted in resistance in pest population, contaminationof soil, water and environment and pesticide residues in the food products (Padmaja Rambabu, 2005).Organochlorine (OC) pesticides are among the agrochemicals that have been used extensively for long periods. They have been used widely in agriculture, as well as, in mosquito, termite and tsetse fly control programs (Guo *et al.*, 2008). OC pesticides are characterized by low polarity, low aqueous solubility and high lipid solubility (lipophilicity) and as a result they have a potential for bioaccumulation in the food chain posing a great threat to human health and the environment globally (Afful *et al.*, 2010). Residues and metabolites of many OC pesticides are very stable, with long half lives in the environment (El-Mekkawi *et al.*, 2009). Studies have shown that DDT is still in its highest concentration in biota of some areas. It is a hydrophobic molecule which disrupts ionic channels like Na+-K+ pumps in nervous cell membrane leading to automatic stimulation of neurons and involuntary contraction of muscles (Esmaili Sari, 2002). Many other recent works have indicated the presence of OC residues in surface waters, sediments, biota and vegetations (Afful *et al.*, 2010; Darko *et al.*, 2009; Adeyemi *et al.*, 2008). The persistent nature of organochlorine residues in the environment may pose the problem of chronic toxicity to animals and humans via air, water and foods intake. Many of these OC pesticides and their metabolites have been implicated in a wide range of adverse human and environmental effects including reproduction and birth defects (Edwards, 1987), immune system dysfunction, endocrine disruptions and cancer (Adeyemi *et al.*, 2008). Fish are used extensively for environmental monitoring (Lanfranchi *et al.*, 2006) because they uptake contaminants directly from water and diet. Generally the ability of fish to metabolize organochlorines was moderate; therefore, contaminant loading in fish is well reflective of the state of pollution in surrounding environments (Guo *et al.*, 2008). In the present study Organochlorine pesticide residues concentration (ppm) were analyzed from the paddy field water samples of Prakasam District.

Study area

Prakasam District occupies an area of 17626 km and average rainfall is 616mm. It is the largest in area among the coastal districts. This district lies between 140 50' 27.725" to 160 17' 21.168" north latitude and 780 31' 1.298" to 80 30' 22.62" east longitude. The average elevation is 10m (30ft). It has a population of above 3054940 as per 2001 census. The total geographical area is 17626 sq.km. It is bounded on the north by Guntur and Mahaboobnagar Districts. On the south by Cuddapah and Nellore Districts, on the east by Bay of Bengal and on the west by Kurnool Districts. The district headquarters is located at Ongole. Many areas in this district depends on ground water for drinking and other purposes. Map of the study area are shown in the following figure1-9.

This research was done to evaluate the level of farmers' pesticides use practiced to rice pest control, their types in the environmental water samples and to propose a sound

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recommendation to minimize the pesticide pollution in the water of Prakasam District.

MATERIALS AND METHODS

Water samples were collected and pooled from six favorite sites of Prakasam District namely Chirala, Kandukur, Kanigiri, Markapur, Giddalur and Cumbum. Samples were collected from surface parts of the paddy field seasonally from winter 2010-11 to autumn 2011-12 following US-EPA (2002). Also, each sampling was carried out in four replicates. Water was collected randomly.

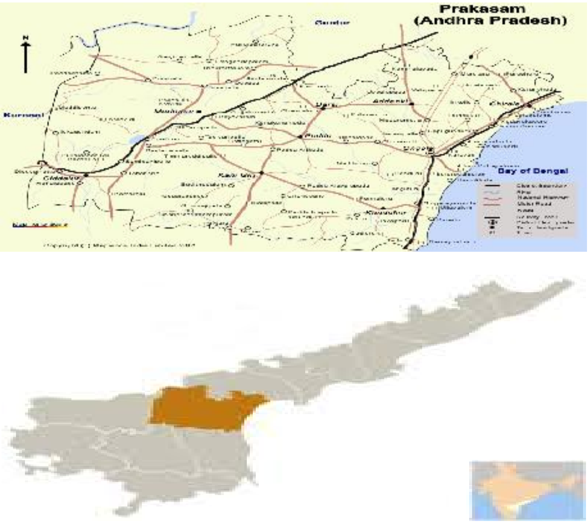

Fig.1 Map of the study area

Sample extraction and Clean up

Extraction

Measured one liter of sample water in 1000 mL of volumetric flask and transferred carefully into two liters Separating Funnel. Similarly measured one liter of de-ionized/HPLC grade water into another two liters separating funnel as a blank. Added 50 g of Sodium Chloride to the sample as well as blank and mixed by shaking the Separating Funnel to dissolve the sodium chloride salt. Added 60mL of Methylene Chloride to the sample and extracted the sample by vigorously shaking the Funnel for 3 minutes with periodic venting to release the excess pressure. After holding for 10-min, organic layer collected. Repeated the extraction two more times with dichloromethane (DCM). Passed the collected extract through the activated sodium Sulphate Column. Evaporated the extracted solution in rotary vacuum evaporator up to 5mL. Evaporated the extract in warm water bath (at about 40^{0}C) volume of 1mL. Injected 1µL to GC-MS. In the laboratory, using liquid-liquid extraction (LLE) as described in APHA (1975).

Clean up

Cleanup was done by column chromatography packed with activated silica gel 10g (2h at 130°C) packed between two layers of sodium sulphate (5g each) and the column was eluted with 150 ml methylene chloride. Eluent was collected and concentrated to dryness. Final samples were prepared in methyl tertiary butyl ether (HPLC grade) and analyzed by Gas Chromatograph.

Sample Analysis

The conditions were maintained for Gas chromatography (GC) are Agilent 7890 A, 5975C MSD model instrument, HP-5MS, 30m x 0.25mm x 0.25 µm 50% Phenyl Methyl Siloxane column, Injection Temp: 260 ^{0}C, Injection Volume 1µl, Column flow: Helium 1mL/min, Oven initial 100 ^{0}C, hold up time 2.0 min, Ramp:70C/min, temperature 290 ^{0}C. For MS, Mass spectrometer with single Quadrupole, Operation in SIM Mode, MS Source: 230 ^{0}C, MS Quad: 150^{0}C Solvent Delay: 3.0 minutes and GC-MSD detector were maintained. The peak area was considered for residue concentration estimation. LOD and LOQ were calculated.

Calculations

All calculations were done as described in USEPA method and the amounts of residues in samples were obtained**.** Recovery tests were carried out prior to the analysis of the samples by fortifying the water sample with standard solutions of organochlorine pesticides to find the efficiency of the analytical techniques. Recovery was in the range of 83-90 per cent for organochlorine pesticides. SPSS statistics software was used for calculations of mean and standard deviation.

RESULTS AND DISCUSSION

Prakasam District wetland is surrounded by farm lands. A large amount of chemicals (fertilizers and pesticides) are used there by farmers which can enter the wetland through running waters and subterranean canals. Also, garbage and wastewaters are poured in the wetland by inhabitants. All of these factors may lead to the contamination of Prakasam District paddy water. Multiple residues of these pesticides in drinking water might have chronic effects due to continuous exposure to levels above MRL in drinking water. Among the different classes of pesticides, organochlorine pesticides are an important class due to their slow decomposition rate, long half-life and high stability in the environment. They are persistent in the environment and accumulate in the upper trophic levels of food chains (Raju *et al*, 1982; Kocan and Landolt, 1989; Gallo and Lawryk, 1991).

Results in the present study have been shown in tables. 1 and 2. The Organochlorine pesticides standard chromatogam is given in figure.10. The Paddy field pooled water (six regions of Prakasam District) samples were analyzed and eight OC residues were traced out. The highest concentration was noticed β-Endosulfan (0.000082 mg/L) followed by γ- HCH (0.000418 mg/L), o,p-DDT (0.000228 mg/L), α-HCH (0.000082 mg/L), δ-HCH (0.000067 mg/L), p,p'-dichlorodiphenyldichloroethane p,p-DDD (0.000062 mg/L), β-HCH (0.000040 mg/L) and Dieldrin (0.000025mg/L). The LOD and LOQ were calculated as 0.000006 mg/L and 0.00002 mg/L.

For beta-endosulfan, Bioaccumulation factor (BAFs) have been estimated using the Estimation Programs Interface (EPI) Suite model (USEPA, 2012a) as described in differet trophic levels. The estimated lower, mid, and upper trophic level BAFs for beta-endosulfan (log KOW = 3.50) are 178.9 L/kg, 199.2 L/kg, and 281.1 L/kg wet-weight, respectively. These estimated BAFs replace the bioconcentration factor (BCF) of 270 L/kg used in the 2002 criteria derivations. Experimental bioaccumulation studies are available for a limited number of fish and shellfish species (USEPA, 2010). In the present analysis, β-Endosulfan concentration in water sample noticed 0.000082 mg/L.

Hexachlorocyclohexane (HCH) consists of eight isomers (Safe, 1997). Out of eight isomers γ- HCH (0.000418 mg/L), α-HCH (0.000082 mg/l), δ-HCH (0.000067 mg/L) Organochlorine residues were noticed. γ- HCH (0.000418 mg/l) noticed as highest concentration out of there HCH residues. The isomers γ-HCH, α-HCH, β-HCH, and δ-HCH were having commercial significance. The pesticide lindane refers to products that contain >99% γ-HCH. The α-, β-, and δ-isomers, as well as technical-grade HCH are not synonymous with γ-HCH (FDA, 1993). Technical-grade HCH is not an isomer of HCH, but rather a mixture of several isomers; it consists of approximately 60–70% α-HCH, 5–12% β-HCH, 10–15% γ-HCH, 6–10% δ-HCH, and 3–4% ε-HCH. FAO/WHO (2003) given guideline value for γ- HCH 0.002 mg/l (2 µg/l), Limit of detection (LOD) value 0.01 µg/l using GC.

DDT is the common name for a man-made chemical, and does not occur naturally in the environment. Chemically, technical DDT is a mixture, the main components of which are p,p' dichlorodiphenyltrichloroethane (p,p'-DDT), (63-77%), o,p'-dichlorodiphenyltrichloroethane (o,p'-DDT) (8-21%), p,p'-dichlorodiphenyldichloroethylene, (p,p'-DDE) (0.3-4%). DDE is also the major metabolite of DDT in biological systems and DDT is rapidly transformed to DDE. Therefore, this assessment will focus on DDT and DDE (IPCS, 1979). DDT and DDE both have low acute systemic toxicity to adult animals, and to humans. DDT isomers o,p-DDT (0.000228 mg/L), p,p-DDD (0.000062 mg/L) residues were traced out in the present study. Compared to Endosulfan and Hexachlorocyclohexane the residue levels were decreased.

World Health Organization (2008) concluded that hypersensitivity to DDT has not been reported, and no conclusive data point to an immunosuppressive effect of DDT exposure. DDT is neurotoxic and causes functional changes in the liver, and at higher dose, liver damage. DDT is unlikely to be genotoxic. DDT causes cancer, notably that of liver, in rodents, and some recent well-conducted studies in humans have demonstrated associations between cancer and exposure to DDT. No mode of action for cancer in rodents has been proposed that was not relevant to humans. DDE has antiandrogenic activity, and DDT was estrogenic. Effects mediated by such hormonal activities have been demonstrated in animals at high doses, but some studies in humans have indicated that they may still be relevant in the human exposure situation. Other than oestrogen/androgen dependent effects, DDT apparently is not teratogenic. FAO/WHO (2001) given guideline value for p,p-DDD 0.001 mg/l (1 µg/l),Limit of detection (LOD) value 0.011 µg/l by GC using ECD.

The resistance of dieldrin to soil leaching generally precludes their appearance in groundwater. The general absence of dieldrin from groundwater samples supports this conclusion (Richard *et al.*, 1975; Spalding *et al.*, 1980). The potential for surface runoff of dieldrin in soils is supported by reports of detectable quantities of these compounds in surface waters (Richard *et al.*,1975). The traced pesticide residues structures ($C_6H_6Cl_6$) were given in figure. 2 to 9.

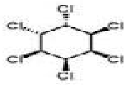

Fig.2 α-HCH

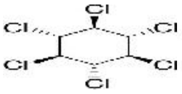

Fig.3β-HCH

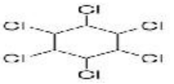

Fig.4 δ-HCH

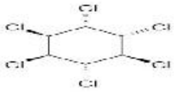

Fig.5 γ-HCH

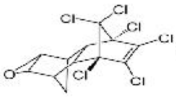

Fig.6 Dieldrin

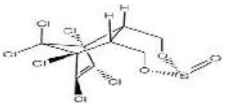

Fig.7 β-Endosulfan

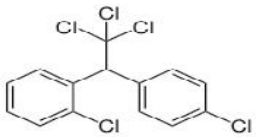

Fig.8 p,p-DDD

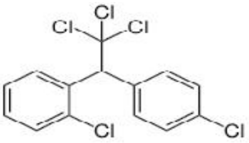

Fig.9 o,p-DDT

Toxicity is estimated by calculating a risk estimate, or the concentration of a substance that presents the least acceptable risk. In the case of cancer-causing toxins, regulations are based on a level of risk that is acceptable, not a safe amount or concentration of a substance (Hayes, 1982; Nash, 1983). The incidence of breast cancer in women is increasing and *in vivo* and *in vitro* studies have shown that organochlorines promote mammary cancer .

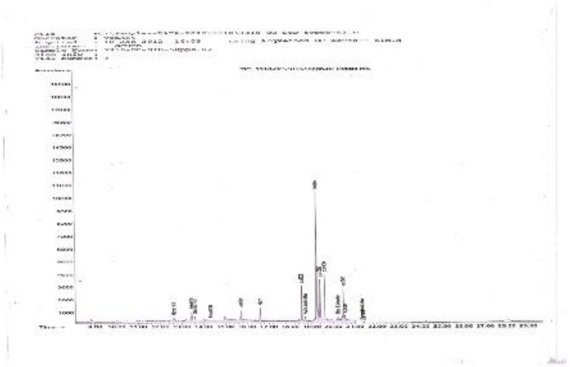

Fig.10 Standard chromatogram of Organochlorine pesticides

Table.1 Retention time and Response of standard concentrations of Organochlorine pesticides.

Compound	R.T	QI on	Response	Conc.	Units	Dev. (Min)
α-HCH	12.633	181	513	50.00	ppb	99
β-HCH	13.448	181	455	50.00	ppb	98
δ-HCH	14.318	181	414	50.00	ppb	97
γ-HCH	13.605	181	505	50.00	ppb	99
Dieldrin	19.368	79	586	50.00	ppb	96
β-Endosulfan	20.184	195	164	50.00	ppb	92
p,p-DDD	20.445	235	2778	50.00	ppb	99
o,p-DDT	20.526	235	427	50.00	ppb	94

Table.2 Pesticide residue levels of organochlorine in water samples of Paddy fields of Prakasam District.

Pesticide	Mean (ppm)	Standard Deviation (SD)	Percentage
α-HCH	0.000082	0.008	88
β-HCH	0.000040	0.002	86
δ-HCH	0.000067	0.004	79
γ-HCH	0.000418	0.006	74
Dieldrin	0.000025	0.012	68
β-Endosulfan	0.000860	0.009	76
p,p-DDD	0.000062	0.005	82
o,p-DDT	0.000228	0.004	86

Mixture of four organochlorines (op' DDT, pp' DDE,b-BHC and pp' DDT) acted together to produce proliferative effects in Michigan cancer foundation (MCF)-7 human breast cancer cells and the combined effect was additive (Gertrudis *et al* 2001). A study suggests that exposure to a mixture of DDT, HCH and endosulfan and decreased fertility in males, an increase in birth defects. Organic chemicals to metabolic disorders that stimulate abnormal production of enzymes (Rao *et al* 1987; Curtis*et al*, 1999). Organochlorine pesticide residues in the water are likely to originate from nonpoint sources via runoff, atmospheric deposition and leaching due to agricultural applications and vector control practices. The water act as a sink for the persistent contaminants, these source may increase pesticide bioavailability and accumulation in the fish.

CONCLUSIONS

In a country like India where sources of food and water are so diverse, prevention of pesticide contamination to the level at which it becomes hazardous or illegal, is preferable to subsequent remediation of excessively contaminated foods and water. There is a strong need to educate farmers, industry, trade and the public about the choice of pesticides and their judicious use. Farmers should be trained to adopt ‘Good Agricultural Practices’. Efforts should be made to promote use of natural pesticides, biological control, and encourage integrated pest management, organic farming and use of safer pesticides. There is a need to make cumulative assessment of risk posed by exposure to multiple chemicals by multiple pathways.

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